CLAIMS

1. A method for preparing a recombinant minimal adenoviral vector stock comprising :

   (a) Introducing in a first cell line (i) a first helper adenoviral vector or virus and (ii) a second helper adenoviral vector or virus the genome of (i) and (ii) comprising 5' and 3' ITRs, a encapsidation region and one or more gene(s) of the early and late regions,

   The genome of (i) deriving from a first adenovirus genome,

   - The genome of (ii) deriving from a second adenovirus genome different from said first adenovirus with the exception of at least the encapsidation region which derives from said first adenovirus genome,
   - Said first helper (i) being capable of packaging said second helper (ii) in said first cell line ;

   (b) culturing the cell obtained in step (a) under appropriate conditions to allow the production of viral particles comprising (ii) and, optionnally (i),

   (c) recovering the viral particles obtained in step (b) from the cell culture,

   (d) introducing in a second cell line said viral particles obtained in step (c) and a recombinant minimal vector,

   (e) culturing the cell obtained in step (d) under appropriate conditions to allow the production of viral particles comprising said recombinant minimal vector, and

   (f) recovering the viral particles obtained in step (e) from the cell culture.

2. The method of claim 1, wherein said first adenovirus is an animal adenovirus and said second adenovirus is a human adenovirus.

3. The method of claim 2, wherein said first adenovirus is a bovine adenovirus and said second adenovirus is a human adenovirus.

4. The method of claim 3, wherein said first adenovirus is BAV3 and said second adenovirus is Ad5.

5. The method of any preceeding claims, wherein said first and/or second helper adenoviral vector is (are) a wild-type adenovirus genome(s).
6. The method of any preceeding claims, wherein said first and/or second helper adenoviral vector is (are) a defective mutant(s) of a wild-type adenovirus genome.
7. The method of claim 6, wherein said first and second helper adenoviral vectors are defective mutants of wild-type adenovirus genomes and are capable of cross-complementing each other for at least one defective function.
8. The method of claim 6 or 7, wherein said first helper adenoviral vector is defective for E1 function.
9. The method of any of claims 6 to 8, wherein said first helper adenoviral vector is defective in E2 function.
10. The method of claim 9, wherein said defective E2 function is caused by a mutation or deletion in at least the gene encoding DBP, Pol and/or pTP.
11. The method of any of claims 6 to 10, wherein said second helper adenoviral vector is defective for E1 function and optionally E3 function.
12. The method of claim 11, wherein said second adenoviral heper vector is an Ad5 genome deleted of nucleotides approximately 455 to approximately 3327 and having nucleotides approximately 149 to approximately 454 comprising the Ad5 encapsidation region replaced by nucleotides approximately 141 to approximately 984 of the BAV3 genome.
13. The method of any preceeding claims, wherein said second adenoviral vector is functional for the E1 function and wherein the E1 region is placed under the control of a non-adenoviral promoter
14. The method of any preceeding claims, wherein said first and second adenoviral helper vectors have an origin of replication recognized by the same E2-encoded gene products.
15. The method of claim 14, wherein the endogenous 5' and 3' ITRs of the first adenoviral helper vector are modified to make the origin of replication recognized by the E2 gene products expressed from the second adenoviral helper vector.
16. The method of claim 15, wherein said modification consists in the replacement of :

- the penultimate 20 bp containing the core origin,
- the penultimate 50 bp containing the entire origin of replication or
- the entire ITRs

of said first adenoviral helper vector by the equivalent sequence of the 5' and 3' ITRs of said second adenoviral helper vector.

17. The method of claim 14, wherein the endogenous 5' and 3' ITRs of the second adenoviral helper vector are modified to make the origin of replication recognized by the E2 gene products expressed from the first adenoviral helper vector.

18. The method of claim 17, wherein the endogenous 5' and 3' ITRs of said second helper adenoviral vector are replaced by the 5' and 3' ITRs of said first adenovirus genome.

19. The method of claim 18, wherein said second helper adenoviral vector is an Ad5 genome deleted of nucleotides approximately 455 to approximately 3327 and nucleotides approximately 28592 to approximately 30470 and having nucleotides approximately 1 to approximately 454 comprising the ITR 5' and the Ad5 encapsidation region replaced by nucleotides approximately 1 to approximately 984 of the BAV3 genome and nucleotides approximately 35826 to approximately 35935 comprising the ITR 3' replaced by nucleotides approximately 34188 to approximately 34446 of the BAV3 genome.

20. The method of any preceeding claims, wherein said first cell line is a non-human cell line.

21. The method of claim 20, wherein said first cell line has a bovine origin and wherein said first adenoviral helper vector is or derives from a BAV3 genome.

22. The method of any preceeding claim, wherein said first cell line is capable of complementing part or all of at least one defective function of said first or second or first and second helper(s).

23. The method of claim 22, wherein said first cell line is complementing the E1 function of said first or second or first and second adenoviral helper vector(s).

24. The method of any preceeding claims wherein said second cell line is of human origin.

25. The method of claim 24 wherein said second cell line is capable of complementing part or all of at least one defective function of said recombinant minimal vector.

26. The method of claim 25, wherein said second cell line is capable of complementing the Ad5 E1 function.

27. The method of claim 26, wherein said second cell line is selected among the group consisting of PER-C6 and 293.

28. The method of any preceeding claims, which comprises more than one amplification step, wherein said viral particles obtained in step (f) are used to reinfect said second cell line in the presence of fresh second adenoviral helper vector or virus.

29. The method of any preceeding claims, which further comprises a purification step of the viral particles obtained in step (f).

30. The method of any preceeding claims, wherein said viral particles obtained in step (f) are substantially helper-free.

31. An animal adenovirus genome having modified 5' and 3' ITRs and wherein said modification consists in the replacement of :

- the penultimate 20 bp containing the core origin,
- the penultimate 50 bp containing the entire origin of replication or
- the entire ITRs

of said animal adenovirus genome by the homologue sequences of the 5' and 3' ITRs of a human adenovirus genome.

32. A viral preparation obtained according to the method of any preceeding claims, wherein said viral preparation is substantially helper-free.

33. A host cell comprising a viral preparation according to claim 32.

34. A pharmaceutical composition comprising a viral preparation according to claim 32 or a host cell according to claim 33.

35. Use of a viral preparation according to claim 32 or a host cell according to claim 33 for the preparation of a medicament for the treatment of diseases by gene therapy or immunotherapy.